The G231N, E232T, N235D polymorphism in peptidylarginine deiminase gene from *P. gingivalis*: a novel factor of virulence and prognosis in periodontitis

In this project we will undertake investigation of the effect of a novel three amino acids polymorphism recently identified in peptidylarginine deiminase from *Porphyromonas gingivalis* on the severity of periodontal disease.

Clinical part of the project will comprise periodontal examination of clinically well-characterized subjects with periodontitis and healthy donors, collection of gingival crevicular fluid, subgingival plaque and blood plasma/serum samples. Collected samples will serve to cultivate *P. gingivalis* strains, and for molecular and biochemical analyses completion. We will evaluate concentration and activity of bacterial peptidylarginine deiminase, prostaglandin E2-dependent pathway and bone resorption markers, and the level of anti-*P. gingivalis* antibodies. Metabolomic analysis will be carried out to detect protein degradation products, re-colonization by periodontal pathogens and disease progression markers. Experimental part will comprise *P. gingivalis* clinical strains genotyping, peptidylarginine deiminase gene sequence analysis, construction of *P. gingivalis* mutant with novel polymorphism and peptidylarginine deiminase purification from mutant. Further, virulence tests of *P. gingivalis* with novel polymorphism will be carried out using gingival fibroblasts and keratinocytes, organotypic model of oral mucosa, monocyte-derived macrophages, osteoblasts, co-cultures of gingival cells with immune cells as well as co-cultures of osteoblasts with osteoclasts. Virulence tests will serve to assess the efficiency of infection, immunomodulatory effect and resorption exerted by *P. gingivalis* harboring polymorphism. Multivariate analyses by employing various cells will enable us to obtain accurate observations and will serve for selection of representative strains harboring polymorphism and those w/o polymorphism for further analyses. Molecular and biochemical tests of *P. gingivalis* with novel polymorphism in peptidylarginine deiminase and enzyme purified from mutant will serve to verify the mechanism of pathogenicity of novel mutant. To this aim peptidylarginine deiminase expression and activity of *P. gingivalis* clinical strains and novel mutant will be carried out. Activity of peptidylarginine deiminase purified from mutant with polymorphism and *P. gingivalis* citrullinome analysis will be evaluated as well. Clinical parameters of periodontium will be correlated with peptidylarginine deiminase concentration and activity, prostaglandin E2 signalling molecules, bone resorption markers, the presence of *P. gingivalis* and the content of low molecular weight metabolites in gingival crevicular fluid, subgingival plaque samples and blood plasma/serum in patients with periodontal disease and control group as well as compared between groups. Multivariate analyses of results obtained in the current project will explain whether the clinical condition of periodontium and local immunity are affected by *P. gingivalis* harboring novel polymorphism in peptidylarginie deiminase.

The impact of polymorphisms in peptidylarginine deiminase from *P. gingivalis* on the severity of periodontitis has not been yet investigated, therefore this topic seems interesting and novel. Confirmation of our preliminary results using abundant cohort of clinically well-characterized patients with periodontitis will undoubtedly have an impact on the gene polymorphism research in periodontology, including polymorphisms of other virulence factors. Taking under consideration that periodontitis is associated with development of some systemic diseases, particularly rheumatoid arthritis, the results obtained in the present project would also have an impact on research aimed to elucidate effect of genetic polymorphisms of human microbiota on rheumatologic disorders. Eventually, if our study fully confirms that the peptidylarginine deiminase polymorphism contributes to pathogenesis of periodontitis, this will have profound therapeutic implications pinpointing inhibitors of this enzyme as a novel and targeted approach to treating periodontitis. Such novel and effective treatment will also have a tremendous economic and social impact.